Lack of Opsonization Potential of 11S Human Secretory γA[1] (36983)

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The secretory γA antibody class undoubtedly contributes significantly to immunological mucosal resistance mechanisms. Viral and bacterial antibody activity has been demonstrated in this class, as have antibodies to dietary antigens, autoantibodies and antibodies against a wide variety of haptens and other immunizing antigens (1–3). Despite the demonstrated significance of this local immunologic system, the mechanism through which its biological function is mediated has to date eluded investigators.

We have studied opsonization as an example of one such possible mechanism. The effect of sensitization of blood group A red cells by purified human colostral γA isoagglutinins in a human monocyte or neutrophil system was compared with the functional activity of serum γM and γG antibodies.

Materials and Methods. Three separate preparations of secretory γA anti-blood group A antibody were isolated from colostral samples obtained from nursing mothers with high titers of anti-A isoagglutinins in serum. Each of these samples was handled separately in the preparative procedure. Colostral samples were centrifuged at 10,000*g*. The supernatant minus the fat, was then passed through a cross-linked blood group. A substance column as previously described (4) after the methods of Moreno and Kabat (5). The antibodies were eluted with the immunodominant sugar, *N*-acetyl galactosamine, dialyzed extensively against 0.85% NaCl, concentrated with Aquacide II (Calbiochem), and subjected to sucrose density gradient ultracentrifugation (10–40%). Only the 11S portions of the gradients were used in the study since the heavier fractions were invariably contaminated with γM (see Fig. 1). The 11S fractions were pooled, dialyzed and concentrated. The concentrations of secretory γA were determined by radial immunodiffusion using an 11S colostral γA standard. The concentrations were also checked by absorbance at 280 nm employing an extinction coefficient of 1.39 (1). The final preparations were tested for possible impurity in radial immunodiffusion with potent anti-γ and -μ chain specific antisera, adjusted to give a lower level of sensitivity of between 10–25 μg/ml.

Serum with high anti-A isoagglutinin titers was treated similarly to colostrum, and the 7S and 19S fractions of the gradient pooled separately. γG and γM concentrations were determined by radial immunodiffusion using pure γG and γM as standards.

White cell preparations. Neutrophils were prepared from blood group A donors' blood samples by the method of Spriggs and Alexander (6), using heparinized blood. Neutrophil-rich preparations were washed in buffered 20% fetal calf serum (FCS) and suspended in 30% FCS. FCS was absorbed with kaolin (4 g/100 ml) to remove nonspecific red cell agglutinins, centrifuged, filtered through a 0.22 μm Millipore filter, and stored in sterile flasks at 4° until used.

Monocyte monolayers were prepared on flying cover slips in Leighton tubes by the method of Cline and Lehrer (7). In some experiments monocyte–neutrophil monolayers were prepared from the whole leukocyte dextran suspensions (7).

Sensitization and phagocytosis experiments. Blood group A red cells were obtained from heparinized blood and washed three times in buffered saline. To 0.02 or 0.04 ml of red cells (at a hematocrit of 50%) was added an equal volume of sensitizing antibody solution. The preparation was incubated in siliconized glass tubes for 1 hr at room temperature. The concentrations of immunoglobulins were ex-

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pressed as micromoles per liter using molecular weights of 1,000,000 for γM and 385,000 for secretory γA (1) in order to make a functional comparison more effective, since each preparation consisted of pure antibody.

Sensitized cells were centrifuged at 1000*g* and resuspended in 0.1 or 0.2 ml Krebs–Ringer buffer (8) containing 2×10^{-5} *M* for calcium and 5.5×10^{-3} *M* glucose. One milliliter of a neutrophil preparation was incubated with 0.1 or 0.2 ml sensitized cells at 37° for 1 hr in siliconized glass tubes. A minimum of 100 neutrophils were counted in wet smears and expressed as the percentage of cells exhibiting attachment or phagocytosis.

To each monocyte monolayer in 1 ml of Krebs–Ringer buffer in 30% FCS on flying cover slips was added 0.02 or 0.04 ml of erythrocytes, sensitized as indicated before, in 0.1 or 0.2 ml of buffer. The preparations were incubated in a moist chamber at 37° for 1 hr. The fluid and nonadherent cells were removed by suction, and the cover slips were washed gently twice with 0.5 ml of buffered FCS at 37°, air-dried and stained with May–Grunwald–Giemsa stain. Enumeration of phagocytosis was performed as for the neutrophils.

Results. When γM was used to sensitize red cells no attachment or phagocytosis by either monocytes or neutrophils was seen at concentrations between 0.001 and 0.14 μmoles/liter. By comparison, γG at a concentration of 0.07 μmoles/liter gave rise to between 7.5 and 17.5% phagocytosis by monocytes. At a γG concentration of 0.16 μmoles/liter 44% phagocytosis by monocytes was induced.

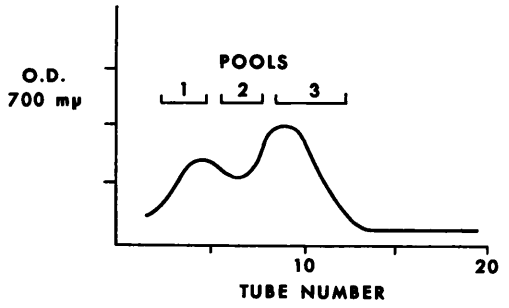

FIG. 1. Distribution of protein eluted from a cross-linked blood group A substance column by *N*-acetyl galactosamine, subjected to 10–40% sucrose density gradient ultracentrifugation. The bottom of the gradient is to the left. Pool 2 contains the 11S antibody.

TABLE I. Monocyte Phagocytosis of γM Anti-A Sensitized Erythrocytes.

γM concn (μmoles/liter)	Serum	% Phagocytosis[a]
0.001	Fresh	20
	Heated[b]	1
0.003	Fresh	43
	Heated	0
0.003	Fresh	81.5
	Heated	1.5

[a] Percentages of cells showing ingestion or attachment of more than 2 red cells.

[b] Heated to 56° prior to use.

When fresh group A human serum was added as a source of human complement, 20% of monocytes showed ingestion of, or attachment to, red cells with γM at a sensitizing concentration of 0.001 μmoles/liter (Table I). At a concentration of 0.003 μmoles/liter between 43 and 81% of monocytes showed binding or ingestion of red cells sensitized with γM in the presence of fresh serum. This phenomenon occurred with both monocytes and neutrophils. Heating of the serum complement source to 56° for 30 min prior to use abolished phagocytosis with both cell types (Table I).

Secretory γA preparations at concentrations between 0.06 and 0.2 μmoles/liter without a complement source did not induce binding or phagocytosis in either neutrophils or monocytes. When fresh serum was added to the system, however, phagocytosis by both neutrophils and monocytes occurred, which was abolished by prior heat inactivation (Tables II and IV).

Since such small amounts of γM antibody had been shown to be active in our system (0.001 μmoles/liter), a rabbit anti-μ chain antiserum was cross-linked to agarose beads by the cyanogen bromide technique (9) and used to absorb the secretory γA preparation. The μ chain antiserum was prepared against the Fc fragment of γM (10) and was rendered monospecific by absorption with γG. After absorp-

TABLE II. Monocyte Phagocytosis of Secretory γA Anti-A Sensitized Erythrocytes.

γA (μmoles/liter)	Serum	% Phagocytosis
0.06	Fresh	15
	Heated[a]	0
0.2	Fresh	63
	Heated	0

[a] Heated to 56° prior to use.

tion of the secretory γA preparation with the cross-linked antiserum binding and phagocytosis were abolished (Table III). Similarly, absorption of a γM preparation with the cross-linked anti-μ antiserum removed all activity.

Different preparations of secretory γA at the same molar concentrations were observed to give highly inconsistent results. The results of another experiment are presented in Table IV in which a much lower functional activity was found with the γA preparation. In this experiment 4000 cells of each type were counted. Absorption with the γM antiserum removed activity both from the γA preparation alone, and a γA preparation to which γM antibody had been added.

Discussion. We have shown in this study that human 11S secretory γA isoagglutinin does not opsonize blood group A red cells for phagocytosis by either monocytes or neutrophils, with or without an added source of complement. No evidence for a γM receptor site was seen in human monocytes in contradistinction to the report of Lay and Nussenzweig (11) with mouse macrophages. Further, pure secretory γA in the present study at several hundred times the minimum effective concentration of γG, and at several times the minimum effective concentration of γM, did not show opsonization capacity or ability to induce binding.

Our conclusion conflicts with a recent report using human colostral γA and blood group B red cells (12), in which evidence for complement dependent opsonization was obtained with both neutrophils and macrophages. The reasons for the disparity are not clear. No absorptions of the γA preparations with a γM immunoabsorbent were apparently performed in the latter study. Since we have shown that such minute quantities of γM antibody can opsonize in a similar system, such absorption experiments must be

TABLE IV. Effect of Absorption with Anti-μ Chain Antiserum on Monocyte and Neutrophil Phagocytosis of γA and γM Sensitized Erythrocytes.

Sensitization with γA (μmoles/liter)	γM	% Phagocytosis[a] Monocytes	Neutrophils
Buffer control		0	0.13
0.19 Unabs.	—	6.5	3.25
0.17 Abs.	—	0.2	0.45
0.19[b] +	0.006 Unabs.	69.5	61.0
0.19[b] +	0.006 Abs.	0.5	0.04

[a] Percentages of 4000 cells. Monocytes and neutrophils were prepared on flying cover slips in Leighton tubes (see Methods).

[b] The antibody preparations were mixed prior to sensitization. Absorption experiments were performed on the mixtures.

TABLE III. Effect of Absorption with Anti-μ Antiserum on Monocyte Phagocytosis of γA and γM Anti-A Sensitized Erythrocytes in the Presence of Fresh Serum.

Sensitization with γA (μmoles/liter)	γM	% Phagocytosis	Antibody concn (μg/ml)
0.2 Unabs.	—	63	77
0.18 Abs.	—	0	66
—	0.003 Unabs.[a]	43	3[a]
—	0.003 Abs.[a]	0	0[b]

[a] These calculations are based on the original concentration of γM antibody before dilution.

[b] This value is assumed on the basis of the functional effects of absorption, and since the concentration of γM was below the level of sensitivity of detection.

considered essential. In our study we have used a pure 11S secretory γA preparation. In the former study there was probably a mixture of polymeric forms. Neither 7S serum γA (13) nor 11S secretory γA fixes complement (14). Recent evidence that myeloma γA, aggregated by chemical means, will activate the complement system through the bypass system (15) has prompted us to examine this with the same γA preparations utilized in the present report. No positive evidence for Cl fixation or bypass activity by 11S secretory γA has been found with this blood group A antigen–antibody system (Colten and Bienenstock, unpublished data).

In our general conclusions, we are supported by the recent studies of Huber *et al.* (16) who were unable to show monocyte binding or ingestion of red cells with secretory γA chemically bonded to their surface. In addition, Eddie, Schulkind and Robbins (17), and Wilson (18) have been unable to show phagocytosis of bacteria sensitized by secretory γA. Further, Lascelles, Gurner and Coombs (19) have not detected γA on the surface of colostral macrophages using the sensitive mixed antiglobulin technique. However, Knop *et al.* (20) have demonstrated *E. coli* phagocytosis by pig colostral γA preparations in a mouse peritoneal macrophage system. As fully discussed in the latter report the levels of impurities in the immunoglobulin preparations might have accounted for the results on the basis of the extraordinarily high biological activity of γM in such a system. It is also possible that polymeric secretory γA may have a different biological function from the naturally occurring 11S form. This possibility would account for some of the differences in those reports reviewed above and the present study.

Further investigations of the possible biological function of the secretory γA molecule are to be encouraged, since despite its undoubted biological significance, the mechanisms of its action remain obscure.

Summary. Pure human colostral 11S secretory γA anti-A isoagglutinin was compared with serum γG and γM as to opsonic activity against A red cells in a system containing either human monocytes or neutrophils. Some preparations of secretory γA had functional activity only in the presence of fresh serum. This activity could be absorbed out by a cross-linked anti-μ chain antiserum. No evidence was found to support the view that 11S secretory γA has an opsonic function in this system.

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